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Distribution and morphometric studies of flagellar sensilla in Emphorini bees (Hymenoptera, Apoidea)

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ABSTRACT

The tribe Emphorini is a group of pollen-collecting solitary bees with a geographical distribution restricted to the western hemisphere. Most of the Emphorini bees collect Page 10 linepollen from a few specific plant families and display specialized behaviors for constructing their nests. Insect sensilla are the basic structural and functional units of cuticle receptors, serving mainly mechano- and chemo-receptor functions. The external morphology of the antennal sensilla has been well characterized in species of different families of Apoidea, however there is scarce information about this issue in solitary bees of the family Apidae. For a better understanding of the association between the external sensory system and several types of behaviors which emerged along the evolutionary history of bees, it is important to characterize the antennal receptors in several representative species of this tribe.

The distribution of the antennal sensilla on the dorsal flagella of 18 taxa was studied in insects of both sexes, using light and scanning electron microscopy. There were six types of sensilla and setae on the antennae, which were identified as sensilla placodea, trichodea, basiconica, coeloconica, coelocapitular and ampullacea. The sensilla trichodea were classified into subtypes, A, B, C-D. Sensilla subtype A were the most abundant sensilla and were distributed over the entire antennae, while sensilla placodea and sensilla trichodea type B, showed a restricted distribution on specific areas of the flagella. We have recognized four patterns of spatial distribution of setae on dorsal flagella. Species having setae on the distal part of the flagellomeres tended to contain a low density of sensilla trichodea type A. Females showed a higher number of sensilla subtypes B and C-D than males; instead sensilla trichodea A were more abundant in males. No significant difference was found in the number of sensilla placodea, ampullacea, coeloconica and coelocapitular. Sensilla basiconica were found only in females. Our results showed that gustative and tactile sensilla were more abundant in female bees, as well as, olfactory receptors predominate in the antennal system of males. The possible coevolution of flagellar sensilla in males and females of solitary bees is discussed in light of previous reports. Patterns of distribution of setae determine the relative abundance of the types of sensilla in the flagellum.

1. Introduction

The tribe Emphorini is a group of pollen-collecting solitary bees with a geographical distribution restricted to the western hemisphere. This tribe includes 10 genera, 5 of which are only found in South America (Roig-Alsina, 1998). Most of the Emphorini bees collect pollen from a few specific plant families (Sipes and Tepedino, 2005) and display a specialized behavior for constructing their nests (Michener, 2007).

Insect sensilla are the basic structural and functional units of cuticle receptors, serving mainly mechano- and chemo-receptor functions (Chapman, 1998). Within the Hymenoptera, the analysis of the external morphology of the sensilla has made important contributions to the studies of the organization of the nervous system as well as to those of the nest-mate discrimination and host-preference behaviors (Ozaki et al., 2005; van Baaren et al., 2007; Nishino et al., 2009). This approach also provides significant tools for taxonomy (Walther, 1983, 1985; Basibuyuk and Quicke, 1999). Recent studies describing inter- and intra-specific phenotypes of antennal sensilla in several genera of a tribe of true bugs, Triatomini, allowed the authors to perform important inferences about the phylogenetic position of the group and habitat selection which are very important issues to be considered when dealing with disease vector insects (Catalá, 1997; Carabajal de la Fuente and Catalá, 2002; Moreno et al., 2006).

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In bees, sensilla have three principal locations: the antennae, the legs and the mouthparts (Galic, 1971; Esslen and Kaissling, 1976; Whitehead and Larsen, 1976). The flagellum is the most important antennal segment carrying sensilla (Wcislo, 1995). It has been reported that olfactory and mechanical signals sensed by the antennal sensilla play a crucial role in mating, foraging, flower recognition and appetitive responses (Kevan and Lane, 1985; Haupt, 2007; Spaethe et al., 2007; Riveros and Gronenberg, 2010). The external morphology of antennal sensilla has been well characterized in species of different families of Apoidea (Ågren, 1989); however there is scarce information about this issue in solitary bees of the family Apidae (Galvani et al., 2008).

For a better understanding of the association of the external sensory system and several types of behaviors which have emerged during the evolutionary history of bees, it is important to precisely characterize the antennal receptors. In this contribution we have studied several representative species of the tribe Emphorini due to the increasing interest in this group of bees which display very specialized behaviors.

2. Materials and methods

2.1. Insects

Live bees were captured in two sites, the biosphere Laguna Oca (Formosa, Argentina) and the reserve Costanera Sur (Buenos Aires, Argentina). Emphorini species were also obtained from the collection of the División de Entomología, Museo Argentino de Ciencias Naturales (Buenos Aires). All the bees analyzed belong to the tribe Emphorini, except for *Chalepogenus caeruleus*, a species from tribe Tapinotaspidini, used for comparative purposes.

2.2. Scanning electron microscopy (SEM)

Antennae were detached from the head and rinsed overnight in 0.4% Triton X100 (v/v) in phosphate buffered saline (PBS: 0.01 M phosphate buffer, 0.13 M NaCl, pH 7.2). Dust was removed from the samples using mild ultrasound treatment of the antennae placed in tubes containing 0.5 ml of PBS. After dehydration in graded ethanol:distilled water series of 20, 40, 60, 90% (v/v) to absolute ethanol for 1 h each, antennae were either critical-point or air dried (Settembrini, 1984). Antennae were further coated with gold palladium and examined in a Philips XL30 SEM microscope. Images were modified only to enhance contrast (Adobe Photoshop, Adobe Systems Inc.).

2.3. Light microscopy

2.3.1. Silver nitrate staining

To detect the presence of pores in the cuticular surface of the sensilla, the antennae were processed following the method of Navasero and Elzen (1991). Briefly, living bees were placed in tubes containing 0.1 M $AgNO_3$ (Biopack, Argentina) in distilled water. After that, they were washed in deionized water, immersed in the photographic developer (Kodak Microdol-X) for 7–10 min and mounted for bright-field microscopy. Also, parasagital sections (12 μm) of antennae processed for silver nitrate staining were obtained with a cryostat (Microm-Zeiss, Waldorf, Germany). Whole-mounted antennae and sections were viewed and photographed using a Nikon E800 microscope equipped with a Nikon DN100 digital-net camera. Images were modified only to enhance contrast (Adobe Photoshop, Adobe Systems Inc.).

2.3.2. Morphology

Antennae were fixed overnight in 2% glutaraldehyde (v/v) in 0.1 M cacodylate buffer, pH 7.2 at 4 °C. The samples were washed in 0.1 M cacodylate buffer, and then post-fixed in 1% osmium tetroxide (v/v) in the same buffer for 2 h. After that, they were dehydrated in graded acetone:distilled water series, followed by 100% propylene oxide, embedded in Epon 862 (Polysciences, USA) for 48 h at 60 °C. One micrometer transverse sections of the flagella were stained with 1% toluidine blue and mounted with Permount (Fisher Scientific, USA).

2.4. Determination of the number of sensilla

The statistical analysis for the average density of sensilla was performed in bees of both sexes of *Ancyloscelis apiformis*, *Ptilothrix relata*, *Diadasina distincta* and *Melitoma segmentaria*. Sensilla were identified and counted from serial SEM micrographs using the Corel Draw software (Corel® Corporation Inc.). Densities of sensilla were calculated by marking a rectangular area on the dorsal side of each flagellomere. In each flagellomere, this area was limited by the entire length of the segment (the *X*-axis) while the *Y*-axis was half the length of each flagellomere (*F*). The seven distal segments were considered in these calculations.

Estimations of the total number of sensilla were calculated according to the method of Fonta and Masson (1982).

2.5. Statistical analysis

Data were analyzed using the GraphPad Prisma Software. Interespecific comparisons were performed by means of non-parametric ANOVA (Kruskall–Wallis test) followed by post hoc comparison using Dunn's test to reveal significant differences between species. In order to evaluate possible differences in the composition of the sensilla between females and males of each species, means were compared with Mann–Whitney non-parametric test. Statistically significant results were differentiated using these symbols: ns, $p > 0.05$; $^{*}0.05 > p < 0.01$; $^{**}0.01 > p < 0.001$; $^{***}p < 0.001$.

2.6. Terminology

The terminology used to describe the antennal morphology and to classify the sensilla follows the Ågren's system (1977), which has been used in most recent studies (Wcislo, 1995; Frasnelli et al., 2010; Shang et al., 2010). This classification is based on the morphological characters revealed by SEM as well as by light and transmission electron microscopy examinations (Ågren and Hallberg, 1996; Esslen and Kaissling, 1976; Whitehead and Larsen, 1976; Slifer and Sekhon, 1961). Dorsal and ventral sides of flagella were also defined according to Ågren' s terminology (Ågren and Sevensson, 1982). Flagellomeres were marked as f1–f11 from a proximal to a distal direction (Fig. 1A and B).

3. Results

3.1. General morphology of the flagellum

The position of the antennae in the head is shown in Fig. 1A. Flagellomeres 2–9 are cylinder-shaped (Fig. 1B and C). The f10 (or the f11 of males) has a wedge-shaped apex wich ends in a rounded tip (Fig. 2A).

3.1.1. Flagellomeres

In all the Emphorini species here analyzed, setae and sensilla trichodea type C-D were the only hairs present on the ventral surface of flagellum. The ventral surface of f10 (f11 in males) showed a few sparse hairs and a smooth surface at the distal end (Fig. 2A). In the remaining flagellomeres, the ventral surface of the cuticle displayed an imbricated pattern (Fig. 2C). The different types of sensilla here described, were located on the dorsal side of the flagellomeres